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Chromosomal distribution of cyto-nuclear genes in a dioecious plant with sex chromosomes

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1   **Chromosomal distribution of cyto-nuclear genes in a dioecious plant with sex**

2   **chromosomes**

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24 **Abstract**

25 The coordination between nuclear and organellar genes is essential to many aspects of

26 eukaryotic life, including basic metabolism, energy production, and ultimately,

27 organismal fitness. Whereas nuclear genes are bi-parentally inherited, mitochondrial and

28 chloroplast genes are almost exclusively maternally inherited, and this asymmetry may

29 lead to a bias in the chromosomal distribution of nuclear genes whose products act in the

30 mitochondria or chloroplasts. In particular, because X-linked genes have a higher

31 probability of co-transmission with organellar genes (2/3) compared to autosomal genes

32 (1/2), selection for co-adaptation has been predicted to lead to an over-representation of

33 nuclear-mitochondrial and nuclear-chloroplast genes on the X chromosome relative to

34 autosomes. In contrast, the occurrence of sexually antagonistic organellar mutations

35 might lead to selection for movement of cyto-nuclear genes from the X chromosome to

36 autosomes to reduce male mutation load. Recent broad-scale comparative studies of N-mt

37 distributions in animals have found evidence for these hypotheses in some species, but

38 not others. Here, we use transcriptome sequences to conduct the first study of the

39 chromosomal distribution of cyto-nuclear interacting genes in a plant species with sex

40 chromosomes (*Rumex hastatulus*; Polygonaceae). We found no evidence of under- or

41 over-representation of either N-mt or N-cp genes on the X chromosome, and thus no

42 support for either the co-adaptation or the sexual-conflict hypothesis. We discuss how our

43 results from a species with recently evolved sex chromosomes fit into an emerging

44 picture of the evolutionary forces governing the chromosomal distribution of nuclear-

45 mitochondrial and nuclear-chloroplast genes.

46

47 **Introduction**

48 The intimate relationships between nuclear and organellar genomes in eukaryotes

49 represent some of the most striking examples of co-evolved mutualisms (Gillham 1994;

50 Lane 2005; Aanen et al. 2014). The long co-evolutionary history of nuclear and

51 mitochondrial genomes is perhaps best illustrated by the finding that the vast majority of

52 mitochondrial genes in animals have been transferred to the nuclear genome (Adams and

53 Palmer 2003; Rand et al. 2004; Burt and Trivers 2006). Indeed, animal mitochondria now

54 encode only a few proteins after having lost the majority of their original genes (Berg and

55 Kurland 2000; Ridley 2000; Bar-Yaacov et al. 2012). Moreover, almost one fifth of the

56 *Arabidopsis thaliana* nuclear genome is of chloroplast origin (Martin 2003), suggesting

57 that organellar-to-nuclear gene movement has played a crucial role in the evolution of

58 plant genetic systems.

59 The evolution of cyto-nuclear interactions and the chromosomal distribution of

60 the genes involved should be influenced by the contrasting modes of inheritance of

61 organellar genes (maternal inheritance) and autosomal genes (bi-parental inheritance).

62 This difference may, for example, result in conflict between nuclear and organellar genes

63 over sex determination and sex ratio (Cosmides and Tooby 1981; Werren and

64 Beukeboom 1998), and several mitochondrial genes in plants are known to cause male

65 sterility (Burt and Trivers 2006; Touzet and Meyer 2014). In systems with XY sex

66 determination, where males are the heterogametic (XY) and females the homogametic

67 sex (XX), genes on the X chromosome spend 2/3 of their time in females (Rand et al.

68 2001) and therefore share a female-biased inheritance pattern relative to Y-linked or

69 autosomal genes, which may result in inter-genomic co-adaptation or conflict.

70   A potential consequence of inter-genomic conflict or co-adaptation between

71 nuclear genes, whose products interact with mitochondrial or chloroplasts (mito-nuclear

72 and cyto-nuclear genes, respectively) and other regions of the genome, is a shift in the

73 chromosomal location of such genes, either becoming more or less abundant on the X

74 chromosome. Several molecular mechanisms have been suggested to be involved in

75 driving gene movement, including gene duplication followed by fixation and subsequent

76 gene loss (Wu and Yujun Xu 2003), and autosomal gene duplications followed by the

77 evolution of sex biased gene expression (Connallon and Clark 2011). The evolutionary

78 mechanisms of this gene movement have also been explored by several recent studies

79 (Drown et al. 2012; Hill and Johnson 2013; Dean et al. 2014; Rogell et al. 2014), and two

80 main processes have been proposed to account for the movement of genes to or from the

81 X chromosome. The co-adaptation hypothesis predicts that the co-transmission of X-

82 linked and organellar genes should result in their co-adaptation, in which selection on

83 beneficial epistatic interactions results in an over-representation of cyto-nuclear genes on

84 the X chromosome relative to autosomes (Rand et al. 2004; Drown et al. 2012). In

85 contrast, the sexual conflict hypothesis predicts the opposite chromosomal distribution,

86 with more cyto-nuclear genes occurring on autosomes to alleviate mutation load in males.

87 To date, empirical evidence for these hypotheses are mixed. Drown et al. (2012) used

88 previously published reference genomes to examine the chromosomal distribution of N-

89 mt genes in 16 vertebrates and found a strong under-representation of such genes on the

90 X chromosomes relative to autosomes in 14 mammal species, but not in two avian

91 species with ZW sex determining systems; note that the co-adaptation hypothesis does

92 not predict that ZW systems should show a bias in the distribution of cyto-nuclear genes.

93   Dean et al. (2014) included seven additional species in their analysis with independently

94   derived sex chromosomes and found that the under-representation of N-mt genes on the

95   X chromosome was restricted to therian mammals and *Caenorhabditis elegans*.

96   Here, we use sex-linked and autosomal transcriptome sequences to investigate the

97   chromosomal distributions of cyto-nuclear interactions in the dioecious annual plant

98   *Rumex hastatulus* (Polygonaceae). Examining cyto-nuclear interactions within a plant

99   species is of interest for several reasons (see Sloan 2014). First, plants carry an additional

100   maternally inherited organellar genome that is absent in animals, the chloroplast genome.

101   This provides an opportunity to compare the chromosomal distribution of two

102   independent kinds of cyto-nuclear interacting genes: nuclear-mitochondrial and nuclear-

103   chloroplast. Second, whereas animal sex chromosomes evolved hundreds of millions of

104   years ago (180 MYA in mammals and 140 MYA in birds; Cortez et al. 2014), the origin

105   of plant sex chromosomes is a more recent event (Charlesworth 2013). In *R. hastatulus,*

106   sex chromosomes are thought to have evolved approximately 15-16 MYA (Navajas-

107   Perez et al. 2005) and genes on the Y chromosome show evidence of degeneration,

108   resulting in a considerable proportion of genes that are hemizygous on the X chromosome

109   (Hough et al. 2014). *Rumex hastatulus* therefore provides an opportunity to test whether

110   the early changes involved in sex chromosome evolution are associated with a

111   concomitant shift in the chromosomal location of N-mt or N-cp genes. Moreover, the

112   presence in this system of X-linked genes that have recently become hemizygous

113   provides an opportunity to compare the chromosomal distributions of X-linked genes that

114   are hemizygous versus those that have retained Y-linked alleles (X/Y genes).

115   Hemizygous genes are particularly good candidates for evaluating evidence for co-

116   adaptation and/or sexual conflict because of their relatively older age (Hough et al. 2014),

117   and because beneficial mutations in such genes are exposed to positive selection

118   regardless of dominance and may therefore spread more rapidly.

119

120   **Methods**

121   *Gene identification and functional annotation*

122   We used sex-linked and autosomal transcriptome sequence data for *R. hastatulus* reported

123   in Hough et al. (2014; GenBank Sequence Read Archive accession no. SRP041588), and

124   obtained three sets of genes with which to test for an over- or under-representation of

125   nuclear-mitochondrial or nuclear-chloroplast genes. In total our analyses included 1167

126   autosomal genes, 624 X-linked genes, and 107 hemizygous X-linked genes. The X-linked

127   and hemizygous X-linked genes were shared between sex chromosome systems in this

128   species (see Hough et al. 2014; *Methods* and *SI Appendix* for full details regarding the

129   identification of such genes from transcriptome sequence data). For autosomal genes, we

130   included those previously identified as confidently autosomal in both *R. hastatulus* sex

131   chromosome systems, as well as those uniquely identified in the XYY system. For each

132   gene set, we queried the sequences translated in all reading frames against the *A. thaliana*

133   protein database using the BLASTx homology search implemented in Blast2GO (Conesa

134   et al. 2005), with a significance threshold (BLAST ExpectValue) of $1x10^{-3}$, above which

135   matches were not reported. We limited our searches to the *A. thaliana* protein database

136   because sequence matches to this database returned more detailed functional information

137   than is available for most other species in the NCBI plant database. We obtained

138   BLASTx results for 1073 autosomal genes (90%), 567 X-linked genes (90%), and 95

6

139   hemizygous genes (89%). Gene Ontology (GO) terms associated with the hits from

140   BLASTx queries were then retrieved using the 'Mapping' function in Blast2GO, which

141   used BLAST accessions to link the queried sequences to functional information stored in

142   the GO database (The Gene Ontology Consortium 2008). Gene names were retrieved

143   using NCBI mapping files 'gene info' and 'gene2accession', and GO terms were assigned

144   to query sequences using the 'Annotation' function with an E-Value-Hit-Filter of $1x10^{-6}$

145   and an annotation cut off of 55 (default parameters). Finally, we ran InterProScan

146   (Quevillon et al. 2005) to retrieve sequence domain/motif information and merged the

147   corresponding annotations with previously identified GO terms. This procedure generated

148   output files containing GO ID's and functional descriptions for each gene in our data set

149   (files will be uploaded to GitHub). The numbers of genes in our final data set with

150   functional annotations and N-mt and N-cp GO annotations are summarized in Table 1.

151

152   *Statistical analyses*

153   We used a similar approach to Drown et al. (2012) and Dean et al. (2014) and estimated

154   the number of N-mt and N-cp genes on the X chromosome and autosomes, and then

155   compared each of these estimates to an expected number. The expected number of N-mt

156   genes was obtained by calculating the product of the proportion of all genes in the data

157   set with mitochondrial annotations (matching GO:0005739) and the number of annotated

158   genes in a given gene set. The expected numbers of N-cp genes were calculated similarly,

159   using GO:0009507. We then calculated the ratios of the observed-to-expected numbers

160   for both N-mt and N-cp genes in each gene set. The observed-to-expected ratio is

161   expected to equal one when there is no under- or over-representation, and greater than

162   one when there is an over-representation. We note that, unlike for X-linked genes, we did

163   not have information regarding the particular chromosome locations for autosomal genes,

164   and therefore could not obtain the expected numbers of N-mt and N-cp genes per-

165   autosome as in previous studies (Drown et al. 2012; Dean et al. 2014). The expected

166   numbers were thus calculated assuming that the set of autosomal genes represented a

167   random sample of the autosomal chromosomes in this species, which is likely a valid

168   assumption given that the sequences were obtained using whole transcriptome shotgun

169   sequencing (Hough et al. 2014). Calculating the expected-to-observed ratios across X-

170   linked, autosomal, and X-hemizygous genes thus allowed us to determine whether any of

171   these gene sets contained an under- or over-representation of N-mt and N-cp genes

172   compared to the expectation based on the proportion of such genes in the full data set. We

173   tested the significance of over- or under-representation using Fisher's exact tests, and

174   calculated 95% confidence intervals for the numbers of N-mt or N-cp genes using 10,000

175   replicate bootstrapped samples. Given our sample sizes of genes with annotations (Table

176   1), Fisher's Exact Tests allowed us to test for differences in the proportions of cyto-

177   nuclear genes on autosomes versus the X-chromosome that were on the order of 5% with

178   ~80% power, whereas power was reduced for smaller differences (Supplementary

179   Material). Similarly, for hemizygous X-linked genes, we calculate that differences of

180   approximately~10% could be detected with ~80% power. All data analysis was done in R

181   (R Development Core Team 2013; scripts will be available for download from GitHub).

182

183   **Results and Discussion**

184   It has been suggested that cyto-nuclear genes may be either over- or under-represented on

185   the X chromosome compared to autosomes, depending on whether their interactions are

186   driven by co-adaptation or sexual conflict (Rand et al. 2001; Drown et al. 2012; Hill and

187   Johnson 2013; Dean et al. 2014; Rogell et al. 2014). We annotated sex-linked and

188   autosomal transcriptome sequences to test these predictions in the dioecious plant *R.*

189   *hastatulus*. We found that neither mitochondria- or chloroplast-interacting nuclear genes

190   were under- or over-represented on the X chromosome (Fisher's exact test, $P = 0.4947$

191   and $P = 0.3074$, respectively; Figure 1). This pattern indicates that neither the co-

192   adaptation nor the sexual conflict hypothesis alone is sufficient to explain the

193   chromosomal distribution of cyto-nuclear genes in *R. hastatulus.*

194   There are several factors that are expected to be important in determining cyto-

195   nuclear gene distributions, and these may explain the lack of bias in *R. hastatulus*. For

196   example, under both the co-adaptation and sexual conflict hypotheses, the age of the sex

197   chromosomes will determine the extent to which selection (either for co-adaptation, or

198   sexual antagonism) has had time to operate, which depends on the rate of gene movement

199   onto and off of the sex chromosomes. Whereas previous studies of cyto-nuclear genes in

200   animals have focused almost exclusively on ancient sex chromosome systems (Drown et

201   al. 2012; Dean et al. 2014; Rogell et al. 2014), our study focused on a dioecious plant

202   species in which sex chromosomes evolved more recently (~15 MYA; Navajas-Perez et

203   al. 2005), and many genes likely stopped recombining much more recently (Hough et al.,

204   2014). The lack of bias in the chromosomal distribution of cyto-nuclear genes may

205   therefore reflect the recent time scale of sex chromosome evolution rather than the

206   absence of biased gene movement. The relatively young age of sex chromosomes may

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207   also have played a role in the lack of bias reported in the sex and neo-sex chromosomes

208   in three-spined stickleback, which evolved ~10 MYA (Kondo et al. 2004) and ~2 MYA,

209   respectively (Natri et al. 2013). Comparative studies of sex chromosomes of different age

210   will be central for understanding the rate at which organellar gene movement occurs.

211   In addition to being evolutionarily older, X-linked hemizygous genes are expected

212   to show a greater effect of over-or under-representation than genes with both X- and Y-

213   alleles because recessive mutations (involved in either co-adaptation or sexual conflict)

214   will be exposed to selection instead of masked by an alternate allele in a heterozygous

215   genotype. We detected a slightly greater under-representation of X-hemizygous N-mt

216   genes compared to autosomes or X-genes with retained Y-alleles, but the effect was not

217   statistically significant ($P = 0.4947$). The opposite pattern was evident for N-cp genes,

218   which were slightly over-represented on hemizygous genes, but again this effect was not

219   significant ($P = 0.3074$). A larger sample of hemizygous genes would be required to more

220   confidently assess whether such genes are in fact more often involved in cyto-nuclear

221   interactions than other genes on the X chromosome, and to test whether the opposite

222   pattern for N-mt and N-cp hemizygous genes is a result of a different rate of nuclear gene

223   transfer between mitochondrial and chloroplast genomes. In particular, the smaller

224   number of hemizygous X-linked genes in our data set implies that power was reduced for

225   this comparison, such that a ~5% difference could only be detected with ~60% power

226   (see Supplementary Material).

227   Another factor that will affect the chromosomal distribution of cyto-nuclear genes

228   is the number of N-mt and N-cp genes that were located on the autosome from which the

229   sex chromosomes evolved. Since the origins of mitochondria and chloroplasts both vastly

10

230   predate that of sex chromosomes (1.5-2 BYA compared to < 200 MYA; Dyall et al.

231   2004; Timmis et al. 2004; Cortez et al. 2014), gene transfer from organellar genomes to

232   the nuclear genome began long before the evolution of sex chromosomes. A bias in the

233   chromosomal distribution of cyto-nuclear genes in either direction may therefore arise if

234   the ancestral autosome was particularly rich or poor in cyto-nuclear genes. Indeed, it is

235   striking that autosomes in the animal species previously examined exhibited extensive

236   variation in the relative number of N-mt genes (see Drown et al. 2012 Figure 1 and Dean

237   et al. 2014 Figure 1 and Figure 2). That the ancestral number of N-mt and N-cp genes is

238   likely to be important is highlighted by the fact that the majority of genes involved in

239   mitochondrial DNA and RNA metabolism in *A. thaliana* are found on chromosome III

240   (Elo et al. 2003). If such a biased autosomal distribution of organellar variation is

241   representative of the ancestral sex chromosomes, the X chromosome could carry

242   significantly more N-mt or N-cp genes because of this ancestral gene number rather than

243   a biased rate of gene movement. This effect is likely exacerbated in early sex

244   chromosome systems, where the majority of genes may not have experienced

245   opportunities for movement. Genetic mapping and comparative genomic studies of genes

246   that have transferred from organellar genomes after the origin of sex chromosomes may

247   provide a means to control for ancestral differences in gene number and provide a better

248   test of biases in organellar-nuclear gene movement.

249   To conclude, our study is the first investigation of the extent to which co-

250   adaptation and sexual conflict have shaped the chromosomal distribution of cyto-nuclear

251   genes in a plant species with sex chromosomes. We found no sign of under- or over-

252   representation of either N-mt or N-cp genes on the X chromosome, implying that neither

253   co-adaptation nor sexual conflict alone can explain the chromosomal distributions of

254   these genes. Instead, we suggest that additional factors, including the age of sex

255   chromosomes and the time that has elapsed since X-Y recombination became suppressed,

256   are likely to have been important determinants of the patterns we observed. To determine

257   whether the lack of under-representation of mito-nuclear genes on the X chromosome

258   reflects an absence of gene movement, future studies should focus on quantifying rates of

259   gene movement after sex chromosome origination, and consider the extent to which

260   neutral processes including the number of mito-nuclear genes on ancestral sex

261   chromosomes have played an important role in shaping the current chromosomal

262   distributions of such genes. Cyto-nuclear conflict and co-evolution have undoubtedly

263   played a major role in many aspects of genome evolution in both plant and animal

264   systems, and the previously reported evidence from therian mammals and *C. elegans*

265   (Drown et al. 2012; Dean et al. 2014) suggests that sexual conflict and co-adaptation

266   might represent important mechanisms driving chromosomal gene movement; however,

267   it remains unclear whether these processes have also shaped the chromosomal

268   distribution cyto-nuclear genes in plants.

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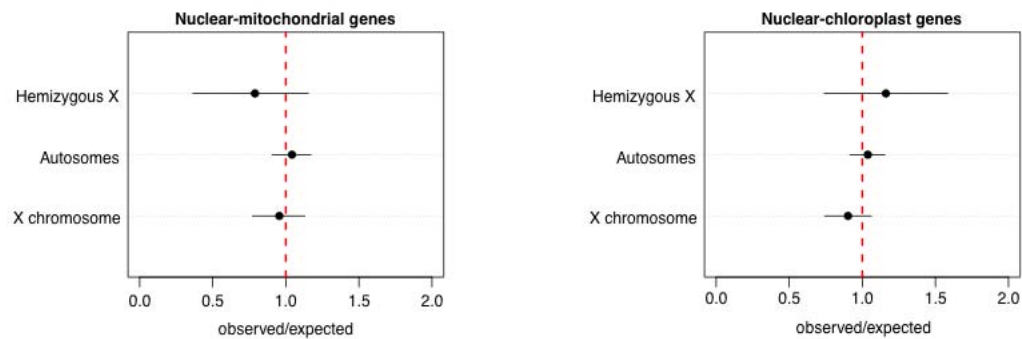
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359 **Figure 1.** Representation of the chromosomal location of cyto-nuclear genes in *Rumex*

360 *hastatulus*. Dots represent the observed to expected ratio of mito-nuclear (N-mt) and

361 chloro-nuclear (N-cp) genes on autosomes, the X chromosome, and hemizygous X genes,

362 with the 95% confidence intervals estimated by bootstrapping (10,000 replicates). The

363 vertical dotted line at 1 represents no over- or under-representation.

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Gene sets	Autosomal	X-linked	X-hemizygous
Original data set	1167	624	107
With annotation	1073	567	95
With N-mt GO ID	194	94	13
With N-cp GO ID	222	102	22

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367 **Table 1.** Number of genes in our data set, including those for which we obtained

368 functional annotations (see Methods) and those with nuclear-mitochondrial and nuclear-

369 chloroplast GO annotations.

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